Recombinant *Phleum pratense* pollen allergen Phl p 4

Clues to new data for an old allergen?

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Introduction

The group 4 allergens of grasses were first described more than 20 years ago and are well known as important major allergens of grass pollen allergy, one of the most common allergies world-wide. Phl p 4 is a basic glycoprotein that, together with Phl p 13, accounts for the high molecular weight fraction of grass pollen allergens. Frequencies of IgE sensitisation higher than 70% have often been reported (1-3), and therefore Phl p 4 seems to be as important as Phl p 5. Contrary to the situation for Phl p 5 and other important Phleum allergens, the primary structure of Phl p 4 has been discovered only recently, despite very considerable efforts in the past.

Fig. 1 Phl p 4 cloning strategy

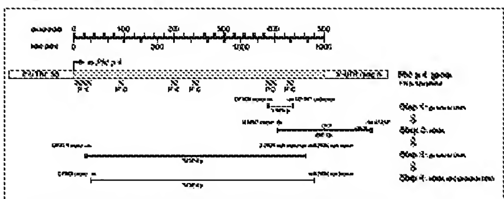
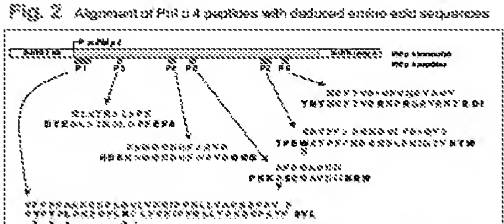


Fig. 2 Alignment of Phl p 4 peptides with deduced amino acid sequences



Results

The experimental procedure that in the end led to the genomic and cDNA sequences of the gene based on a complex PCR strategy involving specific and degenerate primers (Fig. 1). The iden sequence has been confirmed to be Phl p 4 by alignment of the deduced amino acid sequence natural nPhl p 4 derived peptides (Fig. 2). The deduced amino acid sequences of two variants of m Phl p 4 consist of 500 amino acids each, with calculated molecular weights of 56 kDa and basic p 8,8 and 9,2, respectively. A sequence database homology search revealed similarities to berb bridge enzyme-like oxido-reductases (Fig. 3). Recombinant Phl p 4 was expressed in *E. co* inclusion bodies and has been subjected to a refolding procedure. However, the correct folding tu out to be difficult to achieve. Therefore we have expressed Phl p 4 in the methylotrophic yeast *P. pastoris*. The *P. pastoris* derived Phl p 4 is highly soluble and has been purified via His-tag from cu supernatants. Purified recombinant Phl p 4 has been characterised by SDS-PAGE (Fig. 4), inhibition assay (Fig. 5), and protein dots using monoclonal antibodies, as well as IgE conta allergic subjects' sera (Fig. 6). The epitopes of two monoclonal antibodies 3C4, and 5H1 coul localised to the N-terminal and C-terminal domain, respectively (Fig. 7). A 3-D model of Phl p 4 generated on the basis of the vanillyl-alcohol oxidase (VAO) structure (Fig. 8).

Fig. 7 Identification of mAb epitopes

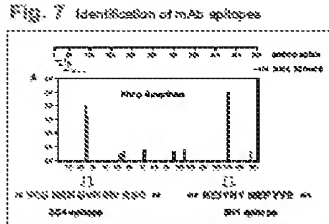


Fig. 8 3-D homology model of Phl p 4

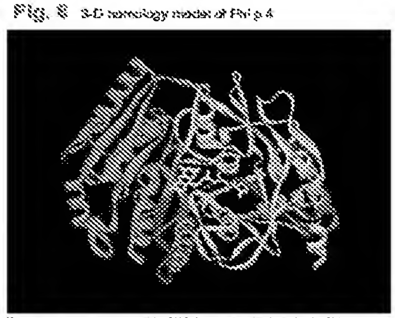


Fig. 3 Alignment of Phl p 4 and the berberine bridge enzyme (BE

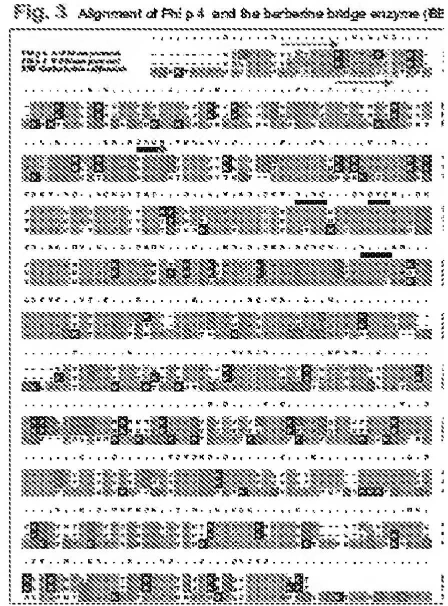


Fig. 4 SDS-PAGE analysis

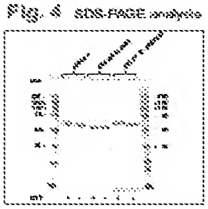


Fig. 5 Human IgE inhibition assay

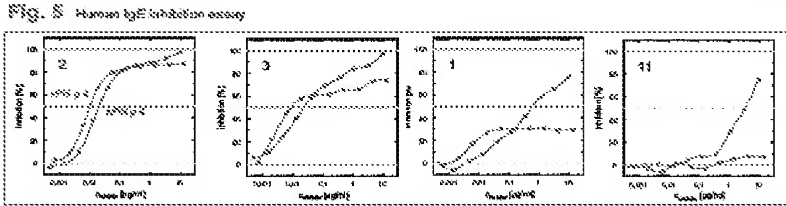
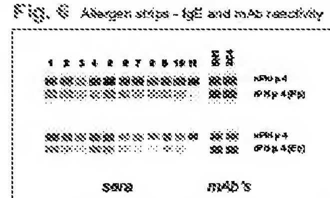


Fig. 6 Allergen strips - IgE and mAb reactivity



Conclusion

References

1) R.E. Rossi et al. (2001), Allergy 56, 1180-1185